Article

*Discovery Medicine* 2026; 38(204): 188–198
https://doi.org/10.24976/Discov.Med.202638204.15

# The Role of BACE2 in Ulcerative Colitis Pathogenesis via the MKP1/NF-$\kappa$B/NLRP3 Pathway

Zan Chen[1], Jingwei Huang[1], Zongshao Yang[1,*]

[1]Department of Gastroenterology, Cangnan Hospital of Wenzhou Medical University, 325800 Wenzhou, Zhejiang, China
*Correspondence: 13858875401@163.com (Zongshao Yang)
Submitted: 13 October 2025 Revised: 28 November 2025 Accepted: 15 December 2025 Published: 20 January 2026

**Background: Ulcerative colitis (UC), a chronic subtype within the spectrum of inflammatory bowel diseases, involves repeated episodes of mucosal injury and impairment of the epithelial barrier. Despite evidence suggesting numerous signaling pathways in UC pathogenesis, the underlying molecular mechanisms remain elusive. Herein, we sought to uncover disease-related genes and investigate the role of $\beta$-site APP cleaving enzyme 2 (BACE2) in disease progression.**

**Methods: Differentially expressed genes were screened from the GSE53306 and GSE75214 datasets. BACE2 expression in a murine UC model was examined using quantitative real-time PCR (qRT–PCR), Western blotting, and immunohistochemistry assays. Functional assays were performed in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages following BACE2 knockdown, including qRT–PCR, Western blotting, enzyme-linked immunosorbent assay (ELISA), Cell Counting Kit-8 (CCK-8) viability assays, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, and flow cytometry. The potential contribution of the MAP kinase phosphatase-1 (MKP1)/nuclear factor kappa-B (NF-$\kappa$B)/NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) pathway was examined. Finally, the effects of BACE2 silencing and MKP1 inhibition on disease progression were evaluated *in vivo* using a mouse model with UC.**

**Results: Bioinformatic analysis identified BACE2 as one of the top upregulated genes in UC datasets. *In vivo*, UC mice exhibited a notable reduction in body mass, an increased disease activity index, a shortened colon length, and increased expression of BACE2 at both transcriptional and protein levels ($p < 0.05$). Histopathological analysis revealed severe epithelial damage and inflammatory cell infiltration, accompanied by enhanced BACE2 expression in colon tissues. *In vitro*, BACE2 knockdown reduced LPS-stimulated tumor necrosis factor-alpha (TNF-$\alpha$), interleukin-1 beta (IL-1$\beta$), and IL-6 production ($p < 0.05$), improved cell viability ($p < 0.05$), and attenuated apoptosis ($p < 0.05$). Mechanistically, BACE2 silencing restored MKP1 expression ($p < 0.05$), suppressed NF-$\kappa$B activation ($p < 0.05$), and inhibited NLRP3 inflammasome signaling ($p < 0.05$). In UC mice, BACE2 knockdown alleviated colonic inflammation, whereas MKP1 inhibition partially reversed these protective effects.**

**Conclusion: BACE2 is aberrantly upregulated in UC and exacerbates disease progression by downregulating MKP1 and activating the NF-$\kappa$B/NLRP3 pathway. Targeting BACE2 may provide a novel approach to inflammatory bowel disease.**

**Keywords:** ulcerative colitis; BACE2; MKP1; NLRP3 inflammasome; inflammation

## Introduction

Ulcerative colitis (UC), a common subtype within the spectrum of inflammatory bowel disease (IBD), represents a long-standing and relapsing inflammatory condition affecting the large intestine, marked by damage to the epithelial barrier, distortion of crypt architecture, and mucosal ulceration [1,2]. Its incidence and prevalence have been increasing worldwide, severely affecting patients' daily functioning and imposing a substantial burden on healthcare systems [3,4]. Although the etiology of UC remains incompletely understood, accumulating evidence suggests that UC pathogenesis arises from a multifaceted interaction among host genetic factors, aberrant immune responses, and environmental influences, as well as imbalances in intestinal microbial communities [5,6]. These factors converge to disturb mucosal immune homeostasis, leading to aberrant activation of inflammatory signaling pathways, which in turn drive persistent intestinal inflammation and tissue injury.

Recent studies underscore the importance of intracellular regulatory molecules in orchestrating the inflammatory response and disease progression in UC [7,8]. One such molecule is $\beta$-site APP-cleaving enzyme 2 (BACE2), a member of the aspartic protease family with diverse biological functions [9–11]. Unlike its homolog BACE1, which is well known for its role in processing amyloid precursor protein in neurodegenerative disease, BACE2 exhibits distinct tissue distribution and has been implicated in multiple physiological and pathological processes. It has been reported to influence pancreatic $\beta$-cell performance and systemic glucose regulation [11], and has also shown to associate with cancer progression, where it influences

cell proliferation, invasion, and apoptosis resistance [12–14]. Beyond metabolic and oncogenic contexts, proteases such as BACE2 may also regulate immune responses by modulating signaling pathways and cytokine activity [15]. Despite these emerging insights, the specific contribution of BACE2 to gastrointestinal inflammation and mucosal homeostasis in ulcerative colitis remains poorly defined.

Mechanistically, inflammation in UC is tightly linked to enhanced nuclear factor kappa-B (NF-$\kappa$B) pathway and subsequent activation of downstream effectors such as the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) signaling complex. This process facilitates the secretion of proinflammatory cytokines and amplifies mucosal injury [16–18]. Importantly, MAP kinase phosphatase-1 (MKP1) serves as a critical inhibitory modulator of mitogen-activated protein kinase (MAPK) and NF-$\kappa$B signaling, thereby modulating inflammatory responses [19,20]. Whether BACE2 contributes to UC progression by regulating MKP1 and consequently affecting NF-$\kappa$B/NLRP3 signaling has not yet been elucidated.

Therefore, this study aimed to delineate the expression profile and biological significance of BACE2 in UC using both bioinformatics analysis and experimental validation in cellular and animal models. Specifically, we explored how BACE2 knockdown affects the release of proinflammatory mediators, apoptosis, and the MKP1/NF-$\kappa$B/NLRP3 pathway activity. Clarifying the role of BACE2 in UC may deepen insights into the molecular mechanisms of intestinal inflammation while identifying BACE2 as a promising therapeutic target.

## Materials and Methods

### *Bioinformatics Analysis*

The GSE53306 and GSE75214 gene expression datasets were obtained from the Gene Expression Omnibus (GEO) repository (https://www.ncbi.nlm.nih.gov/geo/). Differentially expressed genes (DEGs) between UC and control samples were identified using the limma package in R (version 4.2.0, R Foundation for Statistical Computing, Vienna, Austria) with a threshold of $|\log2 \text{ fold change}| > 1.5$ and adjusted $p < 0.05$. Intersecting DEGs between the two datasets were identified using Venn diagram analysis. For subsequent analyses, the ten most significantly upregulated and downregulated genes from each dataset were chosen to further expression and correlation analyses. Heatmaps and correlation matrices were created with the pheatmap and corrplot R packages to visualize these results.

### *Animals and UC Model*

Six- to eight-week-old male C57BL/6 mice were purchased from SPF (Beijing) biotechnology Co., Ltd. and acclimatized for one week. Following acclimatization, mice were randomly divided into four groups (n = 6): (1) Control group, receiving distilled water throughout the study; (2) UC group, administered 3% dextran sulfate sodium (DSS; 9011-18-1, MP Biomedicals, CA, USA) in drinking water ad libitum for 7 days followed by normal water for 3 days; (3) UC + Ad-shBACE2 group, receiving DSS as above described and subsequently treated with Ad-shBACE2 (an adenovirus containing BACE2 short hairpin RNA, Obio, Shanghai, China) via the tail vein; (4) UC + Ad-shBACE2 + MKP1 inhibitor group, receiving DSS and Ad-shBACE2 as above described, along with intraperitoneal injection of the MKP1 inhibitor NSC 95397 (4 mg/kg, dissolved in 200 µL PBS/2.5% DMSO vehicle; HY-108543, MCE, NJ, USA) once daily for 3 consecutive days before euthanasia.

During the experimental period, body weight, disease activity index (DAI), and stool consistency were monitored daily. After completing the 14-day treatment regimen, mice were euthanized, and colon length was assessed, with colonic tissues and blood samples subsequently collected for subsequent histological, molecular, and biochemical analyses. Mice received intraperitoneal anesthesia using pentobarbital sodium (50 mg/kg) before tissue harvesting and were humanely euthanized via $CO_2$ exposure, followed by cervical dislocation to confirm death.

### *Cell Culture and Treatments*

RAW264.7 murine macrophages were obtained from Procell (CL-0190, Wuhan, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, 11965118, Gibco, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, A5670701, Gibco, CA, USA) and 1% penicillin-streptomycin (15140122, Gibco, CA, USA). Cells were maintained at 37 °C in a humidified incubator containing 5% $CO_2$ and sub-cultured every 2–3 days once confluence reached approximately 70–80%. RAW264.7 cells displayed typical macrophage-like morphology, characterized by an adherent, polygonal shape with expanding cytoplasmic extensions. RAW264.7 cells were morphologically normal and tested negative for mycoplasma contamination.

For induction of inflammatory responses, RAW264.7 cells were stimulated with lipopolysaccharide (LPS, L2880, Sigma-Aldrich, MI, USA) at 1 µg/mL for 6 h, while control group received an equal volume of PBS.

For gene silencing, RAW264.7 cells ($2 \times 10^5$ cells/well) were seeded into 6-well plates and transfected with sh-BACE2 plasmids or negative control vector (shNC; GeneChem, Shanghai, China) using Lipofectamine 3000 (L3000001, Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol. Briefly, plasmid DNA and transfection reagent were diluted in medium, mixed for 15 min at room temperature, and the resulting mixture was gently applied to cells. After 6 h, the medium was replaced with refresh complete DMEM. Cells were harvested 48 h later, and the knockdown efficiency was assessed later using qRT–PCR and Western blotting. The target sequence of sh-BACE2 was 5′-

GGTGGACAACCTTCAGGGGGA-3′. A non-targeting sequence (5′-TTCTCCGAACGTGTCACGT-3′) was used as a scrambled negative control.

### *Western Blot Analysis*

Total protein was extracted from colon tissues or cultured cells using RIPA lysis (R0278, Sigma-Aldrich, MI, USA). Protein concentration was determined using BCA assay (PP102-01, Beyotime, Shanghai, China). Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were incubated with primary antibodies against BACE2 (1:500, 16321-1-AP, Proteintech, Wuhan, China), MKP1 (1:1000, 35217, Cell Signaling Technology, MA, USA), NF-$\kappa$B p65 (1:5000, 80979-1-RR, Proteintech, Wuhan, China), p-NF-$\kappa$B p65 (1:2000, 82335-1-RR, Proteintech, Wuhan, China), NLRP3 (1:2000, 30109-1-AP, Proteintech, Wuhan, China), IL-1$\beta$ (1:1000, 26048-1-AP, Proteintech, Wuhan, China), and GAPDH (1:5000, 10494-1-AP, Proteintech, Wuhan, China), followed by HRP-conjugated secondary antibodies (1:2000, SA00001-2, Proteintech, Wuhan, China). Signals were detected using enhanced chemiluminescence (ECL) reagents and quantified using ImageJ software (version 1.53, National Institutes of Health, MD, USA).

### *Quantitative Real-Time PCR (qRT–PCR)*

Total RNA was extracted using TRIzol reagent (15596026CN, Invitrogen, CA, USA) and reverse-transcribed to cDNA using HiScript II Q RT SuperMix for qPCR (R222-01, Vazyme, Nanjing, China). qRT–PCR was performed using TB Green Premix Ex Taq II (638319, Takara, Shiga, Japan) on ABI7500 quantitative PCR instrument (Applied Biosystems, CA, USA). Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. Primer sequences were shown in Table 1.

### *Enzyme-Linked Immunosorbent Assay (ELISA)*

The levels of TNF-$\alpha$, IL-1$\beta$, and IL-6 in culture supernatants were measured using commercial ELISA kits (E-EL-M3063, E-EL-M0037, E-EL-M0044, Elabscience, Wuhan, China) according to the manufacturer's instructions.

**Table 1. Primer sequences.**

| Gene | Primer sequence (5′-3′) |
|---|---|
| *BACE2* | F: GATTGGTGCGACCGTGATGGAA |
| | R: GTTGCTGGCTATGTCTTCCGTG |
| *GAPDH* | F: CATCACTGCCACCCAGAAGACTG |
| | R: ATGCCAGTGAGCTTCCCGTTCAG |

*BACE2*, $\beta$-site APP cleaving enzyme 2; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

### *Hematoxylin-Eosin (HE) Staining*

Colon tissues were collected, gently flushed with chilled PBS to remove fecal residues, and preserved in 4% paraformaldehyde at 4 °C for 24 h. After fixation, tissues were sequentially dehydrated in graded ethanol, cleared with xylene, and embedded in paraffin. Tissue blocks were sectioned at a thickness of 4–5 µm with a rotary microtome, followed by HE staining to evaluate histopathological alterations. Images were acquired with an Olympus BX53 microscope (Olympus, Tokyo, Japan).

### *Immunohistochemistry*

Immunohistochemistry (IHC) was performed to assess BACE2 expression in mouse colon tissues. Paraffin-embedded sections were deparaffinized, rehydrated, and incubated overnight at 4 °C with an anti-BACE2 antibody (1:50, 16321-1-AP, Proteintech, Wuhan, China). Afterwards, sections were treated with HRP-conjugated secondary antibody for 1 h at room temperature, followed by DAB and hematoxylin counterstaining. Images were captured using a light microscope. The quantification of positive staining was analyzed using ImageJ software.

### *Cell Viability Assay*

Cell viability was determined using the Cell Counting Kit-8 (CCK-8, CK04, Dojindo, Japan). RAW264.7 cells ($1 \times 10^4$ cells/well) were plated in 96-well plates and cultured overnight for adherence. After 48 h, 10 µL of CCK-8 reagent was added to wells containing 100 µL of culture medium and incubated for 2 h at 37 °C. Absorbance at 450 nm was recorded with a microplate reader (ELx800, BioTek, VT, USA), and results were expressed as a percentage relative to the control group. Cell viability was calculated using the following formula: Cell viability (%) = (OD experiment – OD blank)/(OD control – OD blank) $\times$ 100%.

### *Apoptosis Assays*

Apoptosis was assessed by flow cytometry. Briefly, cells were harvested, rinsed twice with cold PBS, and stained with Annexin V-FITC/propidium iodide (PI) using a commercial kit (556547, BD Biosciences, CA, USA). Fluorescence signals were analyzed on a flow cytometer (BD FACSCalibur, BD Biosciences, CA, USA), and data were processed with FlowJo software (version 1.53, FlowJo LLC, Ashland, OR, USA).

### *Statistical Analysis*

All experiments were performed at least three times independently. Data are presented as mean $\pm$ standard deviation (SD). The Shapiro–Wilk test was applied to assess data normality before performing parametric analyses. Statistical analyses were conducted using GraphPad Prism 9.5 (GraphPad Software, CA, USA). Differences between two groups were analyzed using Student's *t*-test, and

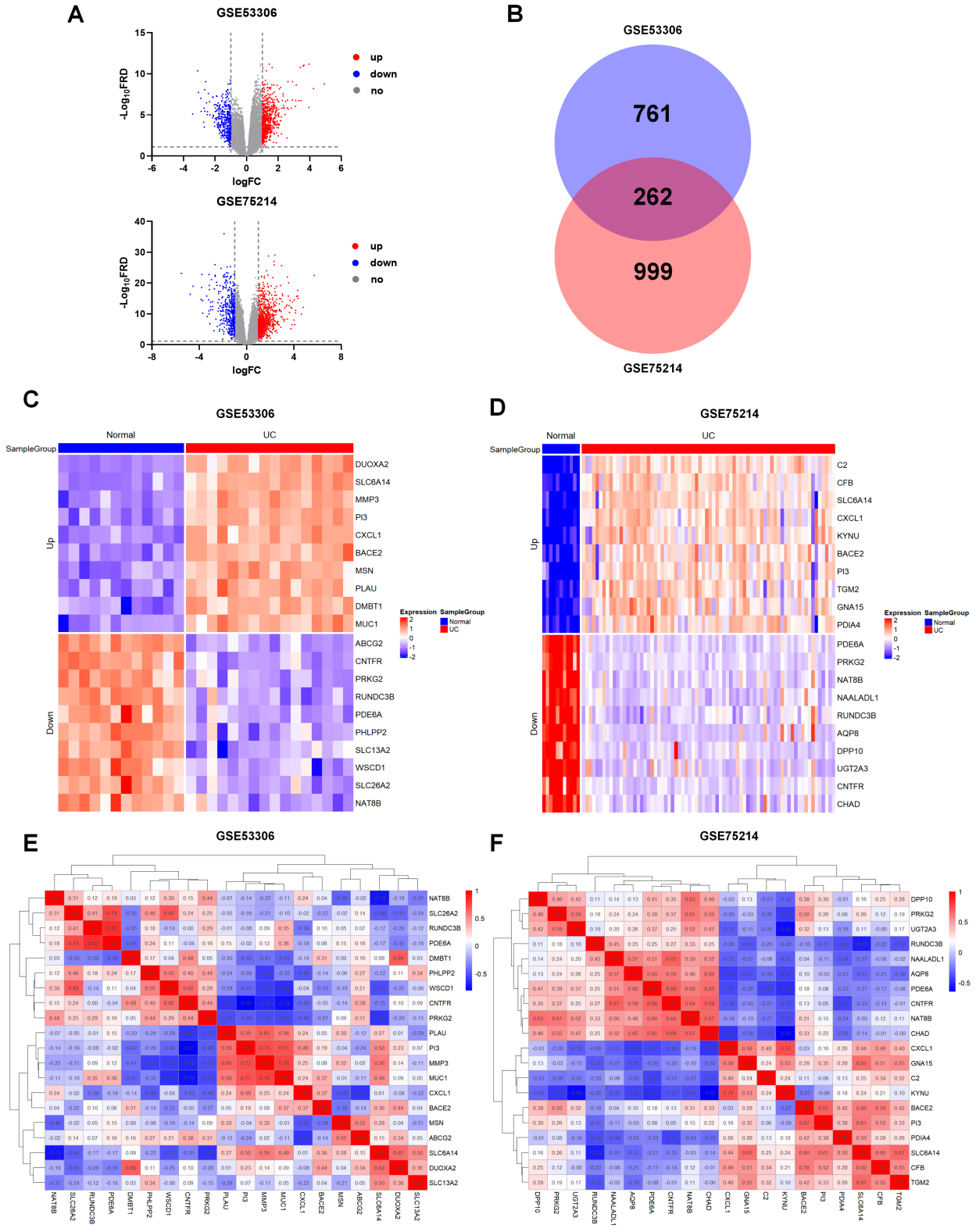


**Fig. 1. Identification and expression analysis of DEGs.** (A,B) Volcano plots of DEGs in the GSE53306 and GSE75214 datasets and the Venn diagram showing their intersection. (C,D) Heatmaps showing the top 10 upregulated and top 10 downregulated DEGs in the GSE53306 and GSE75214 datasets. (E,F) Correlation heatmaps of the top 10 upregulated and top 10 downregulated DEGs in the GSE53306 and GSE75214 datasets. DEGs, differentially expressed genes.

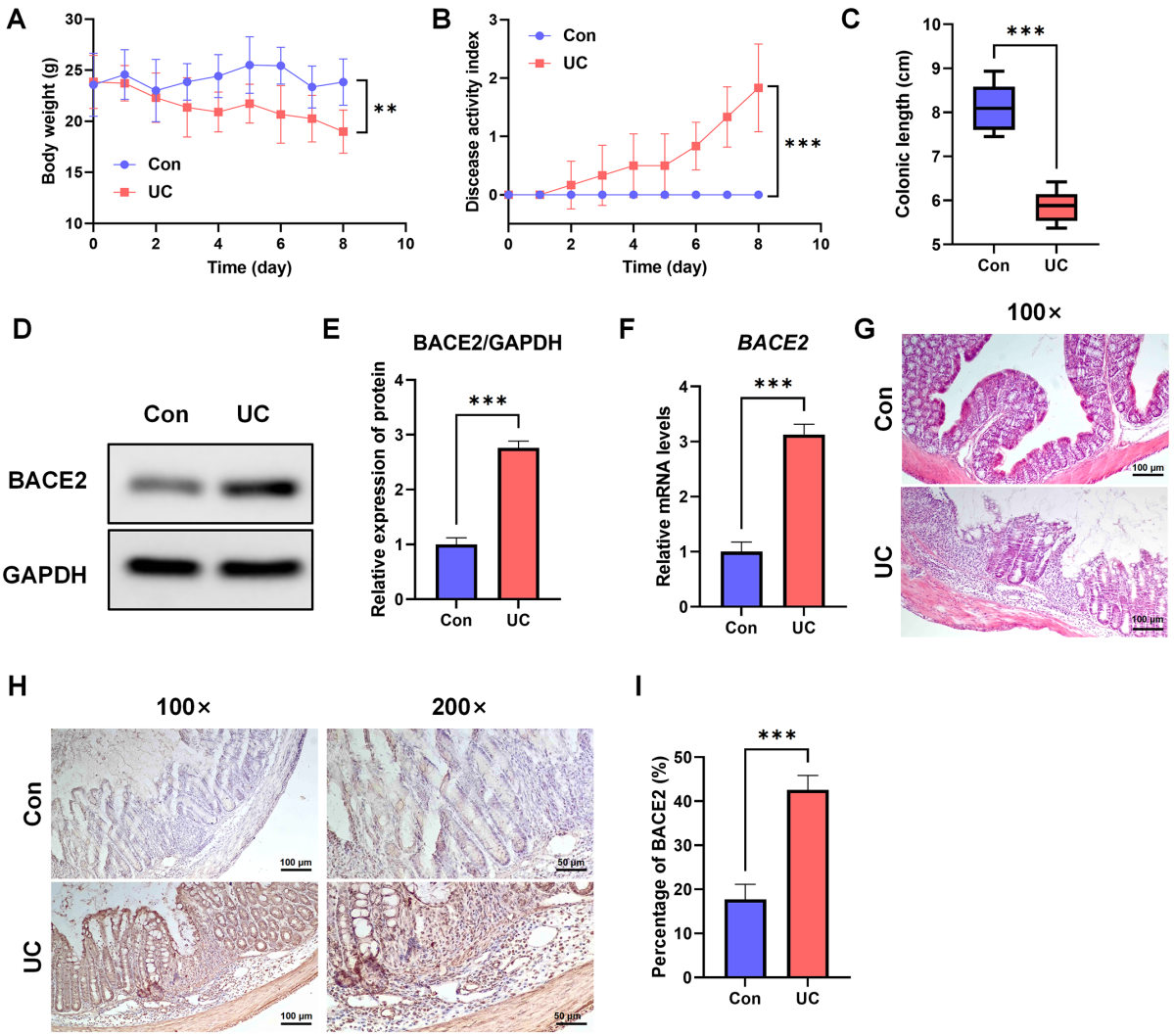


**Fig. 2. Expression of BACE2 in UC mouse models.** (A–C) Changes in body weight, DAI, and colon length in normal and UC mice. (D) Western blot analysis was performed to determine BACE2 protein expression levels in colon tissues. (E) Quantification was conducted to compare relative protein levels between groups. (F) qRT–PCR detection of BACE2 mRNA levels in colon tissues. (G) Histopathological analysis of colon tissues using HE staining. (H) Immunohistochemical staining to assess the expression and distribution of BACE2. (I) Quantification of BACE2 expression based on staining percentage. n = 6. ** $p < 0.01$, *** $p < 0.001$. BACE2, $\beta$-site APP cleaving enzyme 2; UC, Ulcerative colitis; DAI, disease activity index; qRT–PCR, quantitative real-time PCR; HE, Hematoxylin-Eosin.

multiple group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. A $p < 0.05$ was considered statistically significant.

## Results

### *Screening of DEGs in UC Datasets and Identification of BACE2*

To identify potential genes contributing to UC development, RNA expression profiles from the GSE53306 and GSE75214 datasets were analyzed. DEGs were identified in both datasets, as illustrated in the volcano plots (Fig. 1A,B). Intersection analysis revealed 262 common DEGs shared by both datasets. To further characterize these candidates, we examined their expression patterns and identified the most markedly upregulated and downregulated transcripts in each dataset, which are presented in heatmaps (Fig. 1C,D). Correlation analysis of these hub DEGs demonstrated distinct clustering patterns, indicating strong expression relevance among selected genes (Fig. 1E,F). Based on these results, BACE2 emerged as one of the highly expressed genes, suggesting its potential involvement in UC pathogenesis and warranting further functional investigation.

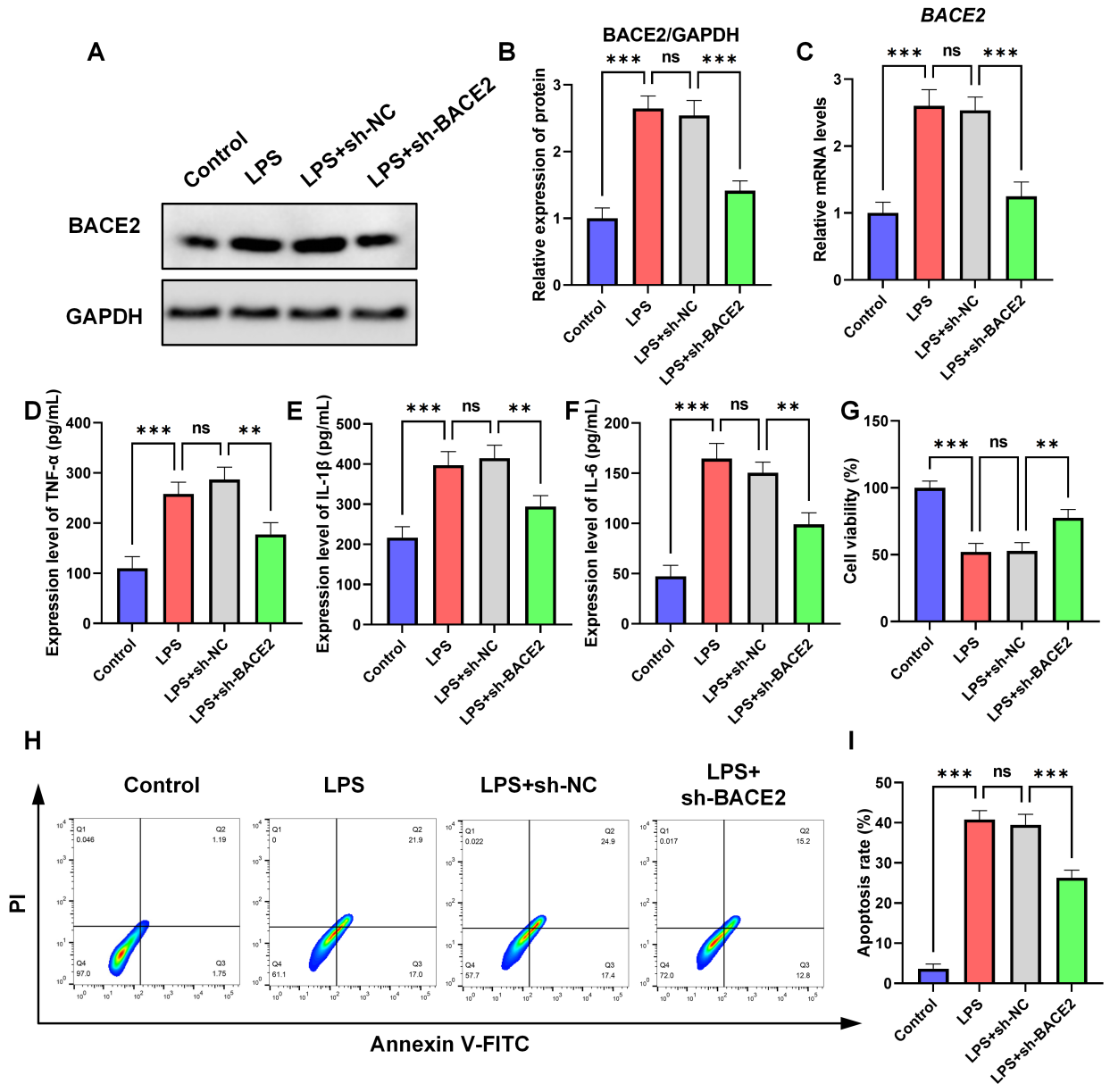


**Fig. 3. Effects of BACE2 knockdown on LPS-induced inflammation and apoptosis in RAW264.7 cells.** (A–C) Western blot and qRT–PCR analyses of BACE2 protein and mRNA expression following LPS and sh-BACE2 treatment. (D–F) ELISA analysis of TNF-$\alpha$, IL-1$\beta$, and IL-6 expression levels. (G) Cell viability analysis by CCK-8 assay. (H,I) Flow cytometry analysis and quantification of apoptosis rates. n = 3. ns $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$. LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay; TNF-$\alpha$, tumor necrosis factor-alpha; IL-1$\beta$, interleukin-1 beta; CCK-8, Cell Counting Kit-8.

### *Upregulation of BACE2 in UC Mouse Models*

A UC mouse model was generated to evaluate the involvement of BACE2 in disease pathogenesis. UC mice displayed significant reductions in body weight, elevated DAI scores, and shortened colon compared with control mice (Fig. 2A–C; $p < 0.05$). Western blotting showed significantly elevated BACE2 protein levels in colon tissues of UC mice (Fig. 2D,E; $p < 0.05$), consistent with the significant upregulation of BACE2 mRNA detected by qRT–PCR (Fig. 2F; $p < 0.05$). Histological examination by HE staining demonstrated typical pathological alterations in UC mice, including epithelial damage, crypt architectural distortion, and inflammatory cell infiltration (Fig. 2G). Moreover, immunohistochemical staining confirmed enhanced BACE2 expression and its altered spatial distribution in UC colon tissues (Fig. 2H,I). Collectively, these findings indicate a significant upregulation of BACE2 in UC mice and suggest its potential contribution to disease progression.

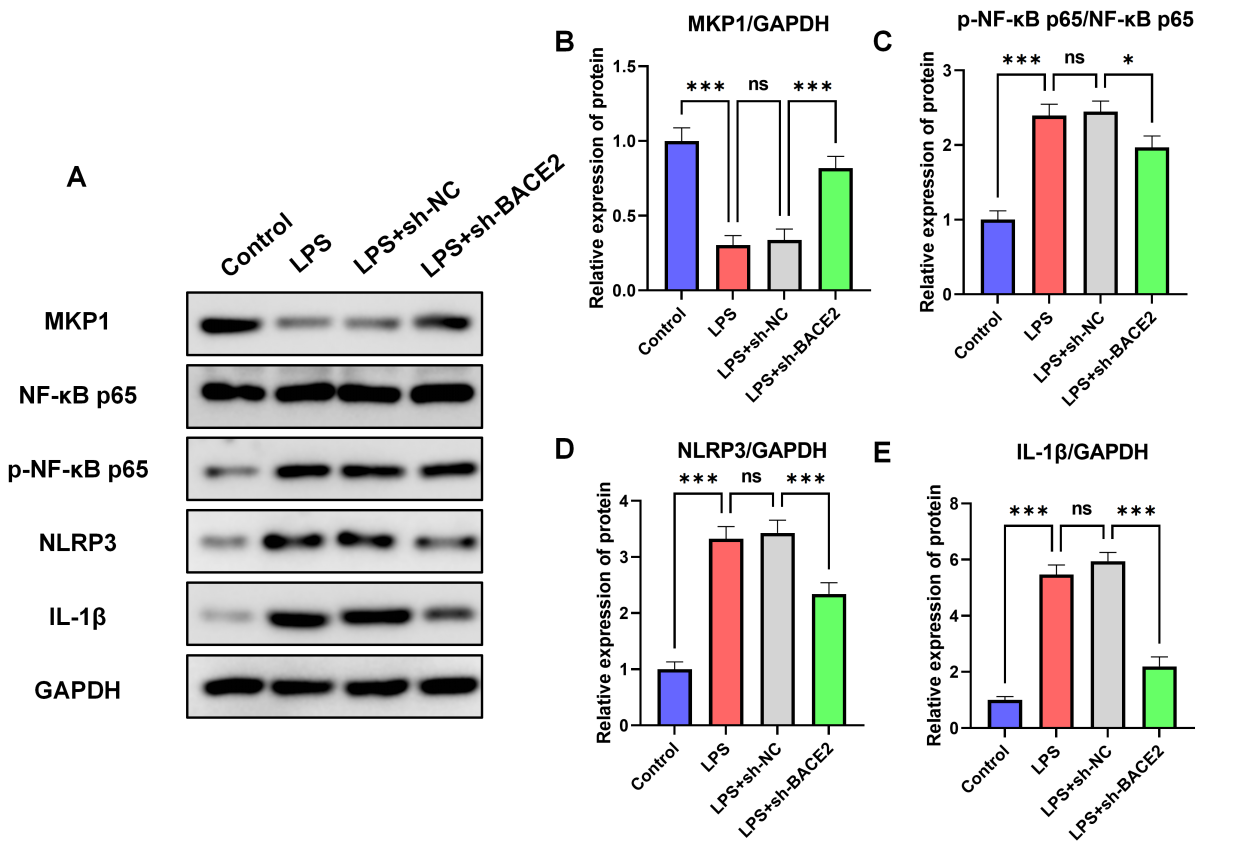


**Fig. 4. Regulation of the MKP1/NF-κB/NLRP3 signaling pathway by BACE2.** (A) Western blot analysis of MKP1, NF-κB p65, p-NF-κB p65, NLRP3, and IL-1$\beta$ protein levels following LPS and sh-BACE2 treatment in RAW264.7 cells. (B–E) Quantitative analysis of MKP1, NF-κB p65, p-NF-κB p65, NLRP3, and IL-1$\beta$ protein expression. n = 3. ns $p > 0.05$, * $p < 0.05$, *** $p < 0.001$. MKP1, MAP kinase phosphatase-1; NF-κB, nuclear factor kappa-B; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3.

### *Knockdown of BACE2 Attenuates LPS-Induced Inflammation and Apoptosis*

To elucidate the functional role of BACE2 in inflammatory injury, RAW264.7 macrophages were exposed to LPS stimulation in the presence or absence of BACE2 silencing. The efficiency of BACE2 knockdown in RAW264.7 cells was first verified by Western blot and qRT–PCR analyses, which confirmed a significant reduction in both protein and mRNA levels in the sh-BACE2 group compared with the control and sh-NC groups (**Supplementary Fig. 1A–C**; $p < 0.05$). Western blot and qRT–PCR analyses confirmed that LPS markedly upregulated BACE2 ($p < 0.05$), whereas transfection with sh-BACE2 effectively suppressed this upregulation (Fig. 3A–C; $p < 0.05$). ELISA analysis demonstrated that knockdown of BACE2 markedly reduced the levels of TNF-$\alpha$, IL-1$\beta$, and IL-6 compared with LPS-stimulated controls (Fig. 3D–F; $p < 0.05$). Functionally, CCK-8 assays revealed that BACE2 silencing alleviated LPS-induced reductions in cell viability (Fig. 3G; $p < 0.05$). Furthermore, flow cytometry consistently revealed that BACE2 knockdown significantly decreased LPS-induced apoptosis in RAW264.7 cells (Fig. 3H,I; $p < 0.05$). Collectively, these findings indicate that BACE2 knockdown attenuates LPS-induced inflammatory responses and apoptosis in macrophages.

### *BACE2 Modulates the MKP1/NF-κB/NLRP3 Signaling Pathway in LPS-Stimulated Macrophages*

To investigate how BACE2 modulates inflammation, we assessed the MKP1/NF-κB/NLRP3 pathway in RAW264.7 cells. Western blotting showed that LPS stimulation significantly enhanced the NF-κB p65 phosphorylation, and upregulated NLRP3 and IL-1$\beta$ levels, while concomitantly reducing MKP1 protein levels (Fig. 4A–E; $p < 0.05$). In contrast, BACE2 knockdown significantly increased MKP1 expression and reversed the above LPS-driven changes (Fig. 4B–E; $p < 0.05$). These findings suggest that BACE2 promotes inflammatory signaling in macrophages at least in part by inhibiting MKP1 and activating the NF-κB/NLRP3 axis.

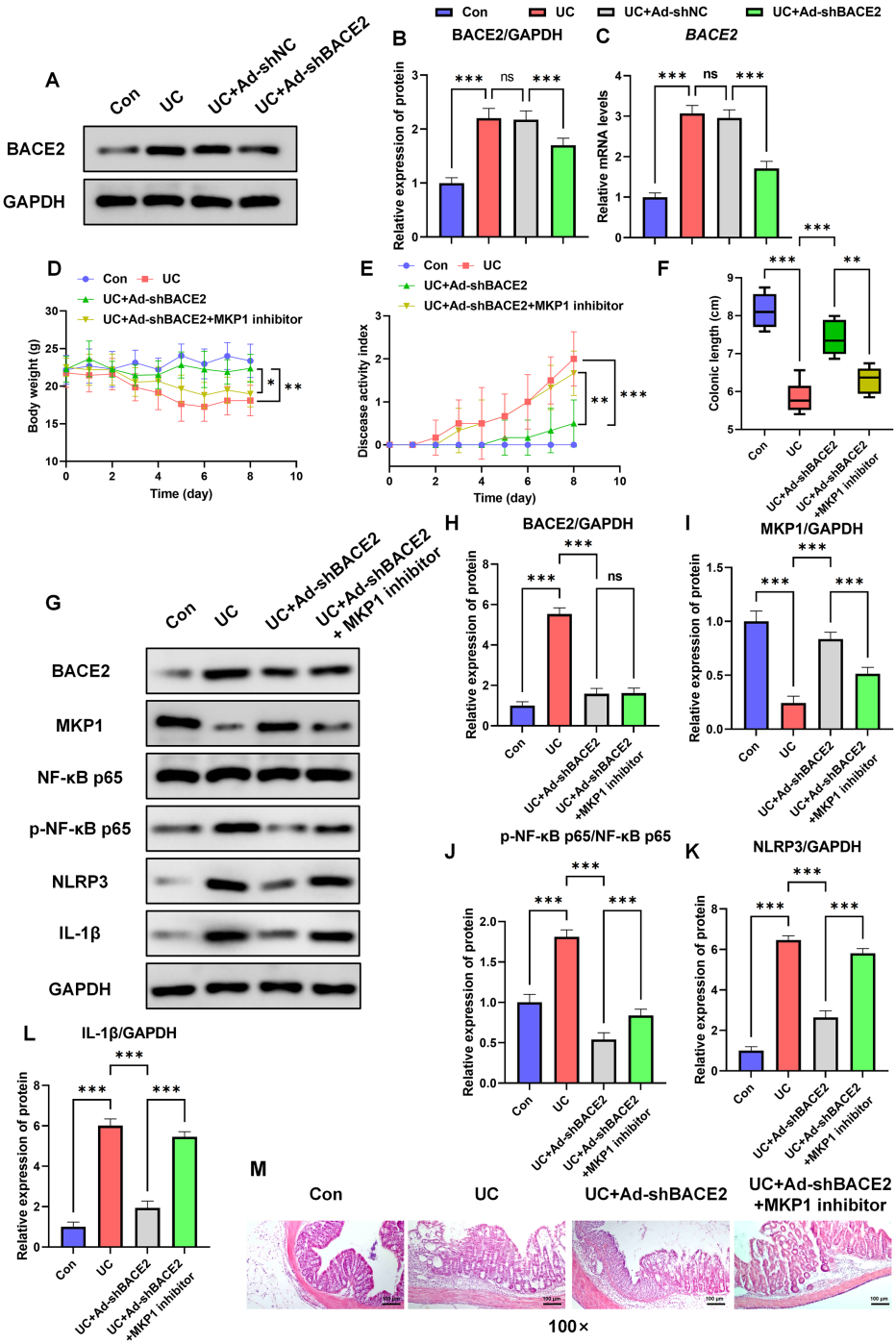


**Fig. 5. BACE2 knockdown and MKP1 inhibition modulate disease progression in UC mice.** (A–C) Validation of BACE2 knockdown efficiency in colon tissues of mice transfected with Ad-shBACE2, determined by qRT–PCR and Western blot analyses. (D–F) Changes in body weight, DAI, and colon length in mice treated with Ad-shBACE2 and MKP1 inhibitor. (G) Western blot analysis of BACE2, MKP1, NF-$\kappa$B p65, p-NF-$\kappa$B p65, NLRP3, and IL-1$\beta$ protein expression in colon tissues. (H–L) Quantitative analysis of BACE2, MKP1, NF-$\kappa$B p65, p-NF-$\kappa$B p65, NLRP3, and IL-1$\beta$ protein expression. (M) Representative HE staining of colon tissues. n = 6. ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### *BACE2 Knockdown Ameliorates UC Progression via MKP1/NF-κB/NLRP3 Signaling in Mice*

To confirm the efficiency of BACE2 knockdown *in vivo*, we first evaluated BACE2 expression in colon tissues of mice transfected with Ad-shBACE2. Consistent with the *in vitro* findings, the efficiency of BACE2 silencing in mouse colon tissues was independently verified in **Supplementary Fig. 2**, where Western blot and qRT–PCR anal-

yses demonstrated significant reductions in both BACE2 protein and mRNA levels in the Ad-shBACE2 group compared with the con and Ad-shNC groups (**Supplementary Fig. 2A–C**; $p < 0.05$). Both qRT–PCR and Western blot analyses showed significantly decreased BACE2 mRNA and protein levels compared with control and UC groups ($p < 0.05$), indicating successful transfection and effective gene silencing (Fig. 5A–C). To further validate the *in vivo* relevance of BACE2 in UC pathogenesis, a DSS-induced UC mouse model was treated with Ad-shBACE2 in the presence of the MKP1 inhibitor. Compared with UC mice, Ad-shBACE2 treatment significantly alleviated disease severity, as evidenced by improved body weight, lower DAI scores, and restoration of colon length (Fig. 5D–F; $p < 0.05$). Western blot analysis further demonstrated that BACE2 knockdown markedly enhanced MKP1 expression while suppressing NF-$\kappa$B p65 phosphorylation, NLRP3, and IL-1$\beta$ levels in colon tissues (Fig. 5G–L; $p < 0.05$). Importantly, the therapeutic effects of BACE2 silencing were partly abolished by MKP1 inhibition ($p < 0.05$), suggesting that MKP1 serves as a key downstream effector of BACE2. Histopathological examination by HE staining revealed that BACE2 knockdown mitigated epithelial disruption, crypt damage, and inflammatory cell infiltration, whereas MKP1 inhibition reversed these protective effects (Fig. 5M). Collectively, these results confirm that BACE2 promotes UC progression by negatively regulating MKP1 and activating the NF-$\kappa$B/NLRP3 signaling pathway *in vivo*.

## Discussion

UC is a chronic inflammatory bowel disorder, characterized by recurrent mucosal injury and epithelial barrier disruption [21]. Despite advances in understanding its immunopathology, the molecular mechanisms driving disease progression remain unclear. This study identified BACE2 as a novel UC-associated gene through integrative transcriptomic analyses of public datasets and further validated its role in both cellular and animal models. The results demonstrate that BACE2 is upregulated in UC and contributes to disease progression by modulating the MKP1/NF-$\kappa$B/NLRP3 signaling axis.

Through differential gene analysis of GSE53306 and GSE75214 datasets, BACE2 was consistently identified among the top upregulated genes in UC samples, highlighting its potential relevance to disease pathogenesis. Functional experiments further revealed that BACE2 expression is significantly increased in UC mouse models, in parallel with severe colonic inflammation and tissue damage. Importantly, BACE2 knockdown in macrophages alleviated LPS-induced inflammatory responses and apoptosis, underscoring its pro-inflammatory role in innate immune regulation. These findings are in line with growing evidence that members of the BACE family exert effects beyond neurobiology: dysregulated BACE2 expression has been associated with altered cytokine milieus and cellular stress responses in non-neural tissues, and proteolytic activity of aspartic proteases can modulate signaling cascades linked to inflammation [13,22].

Mechanistically, our results provide new insights into how BACE2 promotes UC pathogenesis. We observed that BACE2 silencing restored MKP1 expression while suppressing NF-$\kappa$B p65 phosphorylation, NLRP3 activation, and downstream IL-1$\beta$ production. This indicates that BACE2 negatively modulates MKP1, thereby enhancing NF-$\kappa$B signaling and inflammasome activation. Given that MKP1 is a well-established brake on MAPK/NF-$\kappa$B signaling, and its loss promotes sustained intestinal inflammation [23]. Consistent with this, *in vivo* experiments demonstrated that BACE2 knockdown ameliorated UC severity, whereas MKP1 inhibition attenuated these protective effects, confirming the functional interplay between BACE2 and MKP1/NF-$\kappa$B/NLRP3 signaling in disease progression. Similar crosstalk between MAPK phosphatases and inflammasome activation has also been observed in other inflammatory models, underscoring the broader biological relevance of this regulatory axis [24].

Our study thus positions BACE2 as a critical mediator linking epithelial and immune cell dysfunction in UC. Notably, most existing research on BACE2 has focused on its role in neurodegenerative diseases, where it regulates amyloid precursor protein processing. The present findings implicate BACE2 in chronic intestinal inflammation, expanding its biological relevance of beyond the central nervous system and prompting further investigation into its tissue-specific regulation and broader immunomodulatory roles.

Nevertheless, several limitations should be acknowledged. First, while we confirmed the pathogenic role of BACE2 in murine and cellular models, clinical validation using patient-derived tissues is necessary to strengthen translational relevance. Second, the precise molecular interaction between BACE2 and MKP1 remains unclear and warrants further mechanistic exploration, potentially involving protein–protein interaction assays. Finally, given the multifactorial etiology of UC, future research should also assess whether BACE2 interacts with other signaling pathways driving epithelial barrier dysfunction and immune dysregulation.

## Conclusion

This study demonstrates that BACE2 is significantly upregulated in UC and functions as a pro-inflammatory regulator in both cellular and animal models. Silencing of BACE2 alleviates inflammatory cytokine production, restores cell viability, and reduces apoptosis in macrophages. Mechanistically, BACE2 aggravates intestinal inflammation by suppressing MKP1 and thereby promoting NF-$\kappa$B/NLRP3 signaling activation. *In vivo*, BACE2 knockdown ameliorates UC severity, whereas MKP1 inhibition

counteracts these protective effects, highlighting MKP1 as a key downstream mediator. Collectively, these results highlight BACE2 as a critical contributor to UC progression and a potential therapeutic target within the BACE2–MKP1/NF-$\kappa$B/NLRP3 axis.

## Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author Contributions

ZC: Conceptualization, Methodology, Data Curation, Writing — Original Draft. JWH: Investigation, Formal Analysis, Visualization, Writing — Original Draft. ZSY: Supervision, Data Curation, Writing — Review & Editing. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity are appropriately investigated and resolved.

## Ethics Approval and Consent to Participate

All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Cangnan Hospital of Wenzhou Medical University (2024041). All efforts were made to minimize animal suffering and to reduce the number of animals used.

## Acknowledgment

Not applicable.

## Funding

This work was supported by the Wenzhou Basic Scientific Research Project (Grant No. Y20240257).

## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638204.15.

## References

[1] Mauduit A, Mas E, Solà-Tapias N, Ménard S, Barreau F. Main genetic factors associated with inflammatory bowel diseases and their consequences on intestinal permeability: involvement in gut inflammation. Journal of Gastroenterology. 2025; 60: 1323–1338. https://doi.org/10.1007/s00535-025-02289-x.

[2] Khan S, Sebastian SA, Parmar MP, Ghadge N, Padda I, Keshta AS, *et al*. Factors influencing the quality of life in inflammatory bowel disease: A comprehensive review. Disease-a-month: DM. 2024; 70: 101672. https://doi.org/10.1016/j.disamonth.2023.101672.

[3] Solitano V, Bernstein CN, Dotan I, Dignass A, Domilici R, Dubinsky MC, *et al*. Shaping the future of inflammatory bowel disease: a global research agenda for better management and public health response. Nature Reviews. Gastroenterology & Hepatology. 2025; 22: 438–452. https://doi.org/10.1038/s41575-025-01063-x.

[4] Kaplan GG. The global burden of inflammatory bowel disease: from 2025 to 2045. Nature Reviews. Gastroenterology & Hepatology. 2025; 22: 708–720. https://doi.org/10.1038/s41575-025-01097-1.

[5] Liang Y, Li Y, Lee C, Yu Z, Chen C, Liang C. Ulcerative colitis: molecular insights and intervention therapy. Molecular Biomedicine. 2024; 5: 42. https://doi.org/10.1186/s43556-024-00207-w.

[6] Luis Vitetta, Debbie Oldfield, Michael Thomsen. The Intestinal Microbiota: An Environmental Effector for the Endocannabinoid System and Medicinal Cannabis in Inflammatory Bowel Disease. Discovery Medicine. 2025; 37: 1787–1799. https://doi.org/10.24976/Discov.Med.202537200.155.

[7] Tan K, Liu P, Wu Z, Long X, Yu Y, Jiang P, *et al*. Molecular insights into ulcerative colitis and orbital inflammation. Scientific Reports. 2025; 15: 7130. https://doi.org/10.1038/s41598-025-89344-y.

[8] Ning H, Liu J, Tan J, Yi M, Lin X. The role of the Notch signalling pathway in the pathogenesis of ulcerative colitis: from the perspective of intestinal mucosal barrier. Frontiers in Medicine. 2024; 10: 1333531. https://doi.org/10.3389/fmed.2023.1333531.

[9] Yeap YJ, Kandiah N, Nizetic D, Lim KL. BACE2: A Promising Neuroprotective Candidate for Alzheimer's Disease. Journal of Alzheimer's Disease: JAD. 2023; 94: S159–S171. https://doi.org/10.3233/JAD-220867.

[10] Sáez-Valero J, Pérez-González R. BACE2 beyond $\beta$-processing of APP, its neuroprotective role in cerebrovascular endothelium. Journal of Neurochemistry. 2023; 166: 887–890. https://doi.org/10.1111/jnc.15940.

[11] Taylor HA, Przemylska L, Clavane EM, Meakin PJ. BACE1: More than just a $\beta$-secretase. Obesity Reviews: an Official Journal of the International Association for the Study of Obesity. 2022; 23: e13430. https://doi.org/10.1111/obr.13430.

[12] Dimberg J, Shamoun L, Af Geijerstam K, Landerholm K, Wågsäter D. Significance of Gene Polymorphism and Gene Expression of BACE2 in Swedish Patients with Colorectal Cancer. Oncology. 2025; 103: 48–55. https://doi.org/10.1159/000540887.

[13] Farris F, Matafora V, Bachi A. The emerging role of $\beta$-secretases in cancer. Journal of Experimental & Clinical Cancer Research: CR. 2021; 40: 147. https://doi.org/10.1186/s13046-021-01953-3.

[14] He F, Yu J, Yang J, Wang S, Zhuang A, Shi H, *et al*. m$^6$A RNA hypermethylation-induced BACE2 boosts intracellular calcium release and accelerates tumorigenesis of ocular melanoma. Molecular Therapy: the Journal of the American Society of Gene Therapy. 2021; 29: 2121–2133. https://doi.org/10.1016/j.ymthe.2021.02.014.

[15] Tu B, Wu J, Zhang W, Tang H, Dai T, Xie B. A novel cancer-associated membrane signature predicts prognosis and therapeutic response for lung adenocarcinoma. Scientific Reports. 2025; 15: 25482. https://doi.org/10.1038/s41598-025-11105-8.

[16] Zhang J, Xie J, Niu Z, You L, Liu Y, Guo R, *et al*. Ginsenoside Rg2, a principal effective ingredient of *Panax ginseng*, attenuates DSS-induced ulcerative colitis through NF-κB/NLRP3 pathway. Journal of Ginseng Research. 2025; 49: 282–293. https://doi.org/10.1016/j.jgr.2025.02.001.

[17] Cao R, Jing J, Ma Y, Qi W, Huang X, Zhang C, *et al*. Metformin Ameliorates Ulcerative Colitis Through Inhibiting NLRP3 Inflammasome Activation. Journal of Inflammation Research. 2025; 18: 1773–1786. https://doi.org/10.2147/JIR.S503033.

[18] Tamer SA, Köse F, Yanar S, Budak Ö, Bağcı C. Anti-Inflammatory Effects of Spexin on Acetic Acid Induced Colitis in Rats via Modulating the NF-κB/NLRP3 Inflammasome Pathway. Journal of Biochemical and Molecular Toxicology. 2025; 39: e70285. https://doi.org/10.1002/jbt.70285.

[19] Xuan C, Cui H, Jin Z, Yue Y, Cao S, Cui S, *et al*. Glutamine ameliorates hyperoxia-induced hippocampal damage by attenuating inflammation and apoptosis *via* the MKP-1/MAPK signaling pathway in neonatal rats. Frontiers in Pharmacology. 2023; 14: 1096309. https://doi.org/10.3389/fphar.2023.1096309.

[20] Xin Y, Tang L, Chen J, Chen D, Wen W, Han F. Inhibition of miR 101 3p protects against sepsis induced myocardial injury by inhibiting MAPK and NF κB pathway activation via the upregulation of DUSP1. International Journal of Molecular Medicine. 2021; 47: 20. https://doi.org/10.3892/ijmm.2021.4853.

[21] Liang H, Bai X, Zhu R, Song H, Miao Y, Wen Y, *et al*. Diminution of HSP75 disrupts intestinal epithelial barrier by inciting mPTP opening in ulcerative colitis. Cellular Signalling. 2025; 132: 111837. https://doi.org/10.1016/j.cellsig.2025.111837.

[22] Schmidt A, Hrupka B, van Bebber F, Sunil Kumar S, Feng X, Tschirner SK, *et al*. The Alzheimer's disease-linked protease BACE2 cleaves VEGFR3 and modulates its signaling. The Journal of Clinical Investigation. 2024; 134: e170550. https://doi.org/10.1172/JCI170550.

[23] Liu Z, Wang J, Dai F, Zhang D, Li W. DUSP1 mediates BCG induced apoptosis and inflammatory response in THP-1 cells via MAPKs/NF-κB signaling pathway. Scientific Reports. 2023; 13: 2606. https://doi.org/10.1038/s41598-023-29900-6.

[24] He X, Deng B, Zhang C, Zhang G, Yang F, Zhu D, *et al*. HSPA1A inhibits pyroptosis and neuroinflammation after spinal cord injury via DUSP1 inhibition of the MAPK signaling pathway. Molecular Medicine (Cambridge, Mass.). 2025; 31: 53. https://doi.org/10.1186/s10020-025-01086-9.